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Molecular modeling of mechanism of action of anti-*myasthenia gravis* slow-binding inhibitor of acetylcholinesterase

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BACKGROUND: *Myasthenia gravis* (MG) is a chronic autoimmune neuromuscular disorder characterized by fluctuating weakness of voluntary skeletal muscles. The cause of autoimmune response is unknown and only symptomatic therapies for MG are currently available. Pharmacological correction of synaptic failure underlying MG, involves partial inhibition acetyl- and butyrylcholinesterase. Effectiveness of cholinesterase inhibitors in the symptomatic treatment of MG is based on their ability to potentiate the effects of acetylcholine by decreasing the rate of its enzymatic hydrolysis at neuromuscular junctions. Several new inhibitors of AChE were tested in animal model of MG and may be considered as valuable candidates for the treatment of pathological muscle weakness syndromes. In this study, we have investigated mechanisms of ChE inhibition by one of the most active 6-methyluracil derivatives (C547), as well as the possible benefits of using this compound for MG treatment compared to-traditionally used pyridostigmine bromide.

It was experimentally shown that C547 is a «pseudo-irreversible» slow-binding inhibitor of human AChE. Human BChE is reversibly inhibited by C547 with an affinity about 4 orders of magnitude lower than that of human AChE. Slow-binding inhibition of AChE leads to a lasting (over 24 hours) effect on the symptoms of muscle weakness in animal model of MG after a single administration of C547.

OBJECTIVE: The aim of the present molecular modeling study was to reveal mechanism of AChE inhibition by C547 and elucidate its apparent «pseudo-irreversibility».

METHODS: Two principle methods used in the present study were molecular docking and molecular dynamics (MD). Molecular docking was performed with Autodock 4.2.6 software, Lamarckian Genetic Algorithm to obtain structure of protein inhibitor complexes and Local Search for MD snapshots to compare relative binding affinity. For MD simulations NAMD 2.10 software with Charrm 36 force field was used, for the ligand C547 Charmm General Force Field was used, and missing parameters were obtained with quantum mechanical calculations. Unconstrained MD, steered MD (SMD) and free energy calculations with adaptive biasing force were performed.

RESULTS: During unconstrained MD, C547 very rapidly binded to the peripheral anionic site (PAS) of AChE. To pass the bottleneck, application of the external force was required (SMD). Both SMD

modelling and free energy calculation revealed that after crossing the AChE bottleneck, C547 falls into very favorable position. At the same time the rupture of interactions as well as overcoming the bottleneck gates in the course of pulling out procedure requires application of much higher force than during the pulling-in process. This difference between binding and dissociating processes explains apparent «pseudo-irreversibility» of the inhibitor.

CONCLUSIONS: These findings are in good agreement with kinetics study showing that C-547 is a slow-binding inhibitor of type B, i.e. after rapid initial binding of inhibitor, the enzyme-inhibitor complex undergoes an isomerization step. Position obtained by SMD is in good agreement with X-ray data obtained by F. Nachon, IBS, France.

Keywords: Molecular modeling, *myasthenia gravis,* slow-binding, inhibitor, acetylcholinesterase

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